

**128-Plat****Structural Polymorphism of CAG RNA Repeats Investigated by Single-Molecule Mechanical Unfolding**Pan T. Li<sup>1</sup>, William Stephenson<sup>2</sup>.<sup>1</sup>Biological Sciences, University at Albany, SUNY, Albany, NY, USA,<sup>2</sup>Biological Sciences, SUNY College of Nanoscale Science and Engineering, Albany, NY, USA.

Structural polymorphism is an intrinsic characteristic of RNA, especially RNA containing repeated sequences. Transcripts including massively expanded trinucleotide repeats are implicated in many human diseases due to their cellular toxicity. Here we used optical tweezers to unfold RNAs with CAG repeats one molecule at a time. Unfolding trajectories of the RNAs indicated rapid transitions between numerous intermediates with distinctive end-to-end distances, suggesting a flat but rugged folding energy landscape with many shallow local minima. Individual RNAs were nanomanipulated into intermediate structures and their conformational dynamics among local energy minima was monitored in real time. This experimental approach will enable study of other simple sequence repeats, which account for 3% of the human genome.

**129-Plat****How Flaviviruses use a Unique 'Slipknot-Like' Structure to Mechanically Confound a Cellular Exonuclease and Produce Pathogenic RNA**Erich G. Chapman<sup>1</sup>, David A. Costantino<sup>2</sup>, Jennifer L. Rabe<sup>1</sup>, Stephanie L. Moon<sup>3</sup>, Jay C. Nix<sup>4</sup>, Jeffrey Wilusz<sup>3</sup>, Jeffrey S. Kieft<sup>2</sup>.<sup>1</sup>Biochem. and Molecular Genetics, Univ. of Colorado School of Medicine, Aurora, CO, USA, <sup>2</sup>Biochem. and Molecular Genetics, HHMI and Univ. of Colorado School of Medicine, Aurora, CO, USA, <sup>3</sup>Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO, USA, <sup>4</sup>Molecular Biology Consortium, Advanced Light Source, Lawrence Berkeley National Laboratory, Berkeley, CA, USA.

Arthropod-borne flaviviruses (FVs) such as Dengue and West Nile are expanding worldwide health threats. During FV infection, subgenomic flaviviral RNAs (sfRNAs) accumulate in the cell; these sfRNAs are directly linked to pathogenesis and cytopathicity. sfRNAs are formed by the action of conserved RNA structures located at the 5' end of the viral 3' untranslated region (UTR). Remarkably, these structures halt progression of the host-cell's powerful 5' to 3' exonuclease Xrn1, protecting the last ~500 nucleotides of the viral genome from degradation and resulting in the sfRNA. Using a combination of biophysical, biochemical, structural, and virological methods, we characterized the folds and activities of Xrn1-resistant RNAs from Dengue and West Nile viruses and determined the crystal structure of one such RNA (Chapman et al., 2014, eLife; Chapman et al., 2014, Science). We discovered that a specific and complex RNA structure confers Xrn1 resistance, and that this structure likely has biophysical properties unique to this RNA. The structure contains two interwoven pseudoknots in an unprecedented and remarkable fold: the 5'-end of the RNA is threaded through the center of an encircling three-helix junction 'ring', leading to an 'RNA slipknot-like' structure. Modeling of the RNA structure in complex with the Xrn1 enzyme suggests the RNA presents Xrn1 with a mechanical unfolding problem that confounds the helicase activity of Xrn1. These results provide a framework for new experiments aimed at deciphering the detailed biophysical properties used to prevent destruction of this RNA by an enzyme evolved to degrade it.

**130-Plat****Mapping Long Non-Coding RNA Structures with Fragmentation and Next-Generation Sequencing**Jeffrey Viereggs<sup>1</sup>, William Richter<sup>2</sup>, Alex Ruthenburg<sup>2</sup>.<sup>1</sup>Institute for Molecular Engineering, University of Chicago, Chicago, IL,<sup>2</sup>Biological Sciences Division, University of Chicago, Chicago, IL, USA.

Recent years have seen an explosion in our appreciation of the myriad roles that RNA plays in the cell, including the discovery of new classes of regulatory RNAs such as long non-coding RNAs (lncRNAs). The three-dimensional folded structure of many coding and non-coding RNAs plays a key role in determining their function and fate in the cell. Obtaining high quality structural information on large numbers of RNAs is therefore essential, but traditional methods such as crystallography and NMR have been limited due to RNA's rugged folding landscape, charged backbone, and the large size of many RNAs of interest. Chemical mapping, in which folded RNAs are reacted with structure-sensitive chemical probes, provides nucleotide-level resolution mapping of base pairing and backbone flexibility, and can be applied both in vitro and in living cells. Chemical mapping data can be combined with thermodynamics-based structure models to predict RNAs' folded structure, but the confidence of these predictions decreases rapidly with increasing RNA size. Measuring the structure of both the full length RNA and multiple shorter fragments in vitro has been proposed as a route to obtaining high-

confidence structures, but applying this approach to longer RNAs requires high-throughput techniques for readout. Next-generation sequencing (NGS) is well suited to this task, as the large number of reads allows simultaneous analysis of multiple sequences and fragments with multiple chemical agents and the sequencing readout avoids length-dependent inaccuracies associated with gel and capillary electrophoresis. I will present results from applying rational fragmentation with NGS readout to map the structure of the 2.4 kb trans-acting lncRNA HOTAIR and discuss implications for folding pathways of large RNAs and possibilities for in vivo measurement.

**131-Plat****U2 snRNA Conformation is Regulated by Cus2 to Facilitate Dead-Box Protein Loading**

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The spliceosome undergoes dynamic changes in composition and conformation during assembly and splicing. We are interested in studying how the spliceosomal U2 snRNP loads onto pre-mRNA branchsites during assembly. This involves multiple conformations of the U2 snRNA (stem IIa vs. stem IIc), the Prp5 DEAD-box protein, and the Cus2 RRM protein. Our results show that Cus2 binds to WT or stem IIa stabilized model RNAs but not to stem IIc. In contrast, Prp5 binds indiscriminately to all three types of U2 RNA and each equally stimulates Prp5's ATPase. Prp5 appears to either displace or out compete Cus2 for U2 RNA binding in an ATP-independent process. Using single molecule FRET, we show the U2 core RNA is dynamic with interconverting populations of high- (likely stem IIc) and mid-FRET states (likely stem IIa). Addition of Cus2 depletes the high-FRET state, consistent with conformational selection of U2 stem IIa by Cus2. Together, our data support spliceosomal proteins acting in concert to facilitate loading of the DEAD-box onto a particular RNA structure. This represents a new paradigm for protein:RNA interactions that is distinct from ordered binding of structure specific RNA-binding proteins (i.e., ribosome assembly) or from positional control of DEAD-box protein loading during exon junction complex formation.

**132-Plat****RNA Flexibility Depends on Structural Context**

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Nucleic acids require flexibility for essential biological processes such as transcription and translation, among others. The flexibility of unstructured nucleic acids can be difficult to measure using certain structural techniques like x-ray crystallography because of their inherent dynamics. Recent work in our group has used small angle x-ray scattering and single-molecule FRET to measure the properties of short single-stranded homopolymers in solution containing different counterions (1, 2). However, unstructured RNA rarely occurs in isolation. Here, we extend our work by probing the counterion dependence of an RNA molecule containing both structured and unstructured regions. We investigate how the contextual environment of the RNA affects its structure and discuss the potential biological implications.

**References**

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**133-Plat****Single-Molecule Studies of Kissing Loop Interactions in Guanine Riboswitch**

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Guanine riboswitches are structural elements in the 5'-untranslated region of certain mRNAs. These RNA receptors specifically bind guanine and other closely related purines to regulate gene expression. While the basis of ligand-binding and molecular discrimination has been studied to atomic details, the associated structural rearrangements upon ligand-binding are poorly understood. Using a high resolution optical-tweezers that can measure forces at  $\pm 0.15$  pN, distances at 1 nm accuracy with temporal resolution of sub-milliseconds ( $< 0.25$  msec), we followed the structural rearrangements that follows immediately upon guanine-binding at  $< 1$  sec. Our results from the equilibrium sampling and the mutational analysis provide mechanistic insights into the folding of kissing loop interactions that are crucial for the compaction of the riboswitch core leading to the receptor conformation.